Genetic Resources and Crop Evolution **40**: 77–89, 1993.

Isozyme polymorphism and geographic differentiation in a collection of French perennial ryegrass populations

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Received 7 December 1992; accepted 7 April 1993

Key words: Lolium perenne L., isozyme, population genetics, geographic differentiation, environmental factor

Summary

A sample of 60 natural populations of perennial ryegrass from France has been studied for allelic variation at 7 polymorphic enzyme loci. Population genetic statistics are of the same magnitude than those previously reported for other outbreeding, short-lived perennial species (P = 64%, A = 2.75, H = 0.270).

Genotype frequencies at most collection sites do not deviate significantly from Hardy-Weinberg expectations, with however a slight deficit of heterozygotes which may be accounted for by a Wahlund effect. Gene diversity is mainly explained by the within population component. The between population differentiation F_{st} averaged on 4 loci is only 0.054, which accounts for only 6% of the whole diversity.

When mapped, most allele frequencies do not show any special structure. Only five alleles present a clinal trend from North to South. These 5 alleles are probably related to some climatic factors such as average temperature or potential evapotranspiration. The causal hypotheses about the low level of between-population differentiation and spatial structure are discussed with reference to the literature.

The consequences of the found population structure for sampling and conservation strategies of natural populations for genetic resources are presented and discussed.

Introduction

Genetic variation for quantitatively inherited traits controlling morphology and development enables plant populations to grow in a range of environments. In widespread plant species, this variation is often spatially structured, either in response to selection caused by differences in environmental factors, or as a consequence of random drift and isolation-by-distance (Sokal, 1986). Knowledge of genetic population structure, patterns of geographic variation and the influence of selective agents should be taken into account in sampling and conservation strategies of natural populations (as germplasm for present and future breeding use).

Lolium perenne L. (perennial ryegrass) is a widespread forage grass commonly used in agriculture of temperate countries. Ecotypic differentiation for agronomic traits has been reported e.g. Tyler et al. (1984), Falcinelli et al. (1988), Charmet et al. (1990). Associations between agronomic traits and environmental factors have been reported by Balfourier & Charmet (1991).

Variation at isozyme loci is generally considered as selectively neutral (Kimura, 1983), and therefore provides a tool for estimating divergence caused by genetic drift. In some instances however, allelic frequencies of isozymes have shown to be correlated with environmental factors, thus probably reflecting selection pressure (Nevo et al., 1986). It is therefore necessary to assess the neutrality of

alleles before using them as genetic markers of stochastic processes such as random drift and isolation-by-distance.

In perennial ryegrass, isozyme techniques were first developed by Hayward & Mcadam (1977), who determined the manner of inheritance of a number of isozymes. Further studies of Mendelian genetics of isozyme loci have been carried out by Nielsen et al. (1985), Ostergaard et al. (1985), Polans & Allard (1985), Lallemand et al. (1991). Most of these studies deal with cultivar identification or stability.

Surveys of isozyme variation in natural populations of forage grasses have been reported for ryegrass (Hayward, 1985; Arcioni et al., 1988; Oliveira & Charmet, 1989), cocksfoot (Lumaret, 1984), red fescue (Livesey & Norrington-Davies, 1991), *Cynosurus cristatus* L. (Ennos, 1985), and *Arrhenaterum elatius* (L.) J. et C. Presl (Ducousso et al., 1991).

This paper describes a survey of allelic variation at 7 polymorphic isozyme loci on a sample of wild French populations of perennial ryegrass. A first attempt to describe geographic patterns of variation has been made by mapping allele frequencies, while the relationships between allele frequencies and environmental factors have been studied through multivariate methods.

Materials and methods

Sixty populations of perennial ryegrass were sampled from a collection of 550 populations from France described by Charmet et al. (1990). The choice took into account both a hierarchical clustering based on agronomic traits and ecogeographic factors such as natural region and habitat as reported by Balfourier & Charmet (1993). Populations were taken from every cluster, with a number approximately proportional to the logarithm of cluster size as suggested by Brown (1989). The sampling strategy also aimed that every type of habitat and every administrative region be represented in the sample.

Starch gel electrophoresis was carried out on 10 leaves of 4–6 weeks old seedlings with an average sample size of 98 plants per population (range 75-144). Six enzyme systems were revealed on slices of a single lithium-borate gel (Hayward & Mcadam, 1977): phosphoglucoisomerase (PGI, EC 5.3.1.9), Acid phosphatase (ACP, EC 3.1.3.2), Glutamate-oxaloacetate-transaminase (GOT, EC 2.6.1.1), Superoxide dismutase (SOD, EC 1.15.1.1), Peroxidase (PRX, EC 1.11.1.7) and Isocitrate dehydrogenase (IDH, EC 1.1.1.42). Enzyme extraction, electrophoresis and enzyme assays were carried out according to the procedures of Hayward & Mcadam, (1977), with staining recipes from Pollans & Allard (1985) for PRX and Greneche et al. (1991) for IDH. These 6 systems lead to 7 polymorphic loci (GOT giving Got-2 and Got-3).

Data of individual genotypes have been stored in an ORACLE-developed database (Ravel et al., 1992), and further analysed using BIOSYS 1 (Swofford & Selander, 1981) for population genetics parameters. S (Becker et al., 1988) was used for graphic representations of the allelic frequencies in maps, and SAS (SAS Institute, 1988) for the factorial correspondence analysis.

The following population genetics statistics have been computed: mean number of alleles per locus (A), proportion of polymorphic loci at the 0.99 criterium (P), average heterozygosity expected under panmixia (H), Wright's F-statistics (Wright, 1965, Nei, 1977): F_{it} (overall), F_{is} (within population) and F_{st} (between populations). F_{it} and F_{is} are the fixation indices of individuals relative to the total (=entire) population and its subpopulations (i.e. each of the 60, averaged), respectively. These fixation indices measure the deficit of heterozygotes $(Ht - Ho)/Ht$, where Ho is the observed number and Ht the expected number of heterozygotes under panmixia. The basic relationships is:

$$1 - F_{it} = (1 - F_{is})(1 - F_{st})$$

Then the between populations component of differentiation F_{st} was decomposed through a hierarchical F analysis (Wright, 1978) by introducing a between cluster F_{xt} and a within cluster F_{sx} index. Three factors of classification have been used as x: agronomic cluster (Charmet et al., 1990), administrative region and the type of habitat. The basic formula then becomes:

$$1 - F_{it} = (1 - F_{is})(1 - F_{sx})(1 - F_{xt})$$

The average number of migrants between populations have been estimated from F_{st} as $Nm = (1/4)[(1/F_{st}) - 1]$, according to Slatkin & Barton (1989).

The relationships between isozyme frequencies and environmental factors (mostly climatic data) have been investigated through multiple factorial analysis as described in Lumaret (1984). Allele frequencies were split in to 3 qualitative classes of nearly equal numbers and used as active variables. The following climatic traits, also split into 3 classes, were used as additional variables:

- –Average annual temperature (mean T)
- –Average minimum temperature of the coldest month (min T)
- –Average maximum temperature of the warmest month (max T)
- –Average potential evapotranspiration during the growth season (etp).

Class limits are given in Table 1. Factorial correspondence analysis uses the Chi-2 metric to estimate a proximity between an allele frequency class and a climatic data class, in the space generated by the factors. These proximities can be illustrated by plotting frequency and climatic classes with the first two factors as x- and y-axis. Allele frequency and climatic classes closely located can be interpreted as a "correspondence" i.e. a non-random association. This method is powerful in identifying non-linear relationships between qualitative or quantitative traits (after transformation into a "categorized" variable).

Table 1. Limits of the classes of allele frequencies and climatic traits for the factorial correspondence analysis

	Class limits	No of populations
acp-a (1)	$0.20 \ll 0.38$	20
acp-a (2)	$0.38 \ll 0.50$	20
acp-a (3)	$0.50 \ll 0.78$	20
acp-b (1)	$0.14 \ll 0.40$	20
acp-b (2)	$0.40 \ll 0.53$	20
acp-b (3)	$0.53 \ll 0.73$	20
Got 3-c (1)	$0 \ll 0.05$	19
Got 3-c (2)	$0.05 \ll 0.13$	21
Got 3-c (3)	$0.13 \ll 0.33$	20
Got 3-d (1)	0	
Got 3-d (2)	$0 \ll 0.02$	19
Got 3-d (3)	$0.02 \ll 0.11$	19
Prx-a (1)	$0 \ll 0.01$	20
Base a (0)	$0.01 \ll 0.04$	20
Prx-a (3)	$0.004 \ll 0.20$	20
latitude (1)	$42.8 \ll 45.7$	20
latitude (2)	$45.7 \ll 48.1$	20
latitude (3)	$48.1 \ll 50.7$	20
mean T (1)	$6.4 \ll 9$	11
mean T (2)	$9 \ll 12$	39
mean T (3)	$12 \ll 15.3$	10
min T (1)	$-7 \ll -1$	24
min T (2)	$-1 \ll 7$	15
min T (3)	$7 \ll 13$	21
man T (1)	$19 \ll 22$	19
man T (2)	$22 \ll 24$	15
man T (3)	$24 \ll 31$	26
etp (1)	$575 \ll 625$	24
etp (2)	$625 \ll 675$	16
etp (3)	$675 \ll 1075$	20

Results

Genetic structure

Table 2 gives a summary of allelic variation and average heterozygosity found in this collection of 60 ryegrass populations. Data for individual populations cannot be presented extensively, but are available on request.

A total of 28 alleles have been found, Pgi and Acp being the most polymorphic loci, with up to 6 alleles, while Sod, Prx and Idh show a very frequent b allele and one or two much rarer variants with the electrophoretic technique employed. Of 28 alleles, 12 can be considered as common widespread according to the classification of Brown (1978), 8 as rare widespread (mean frequency less than 5% but presence in more than half of the populations), and 8 as rare and sporadic. Four alleles of this last category reach a frequency of 5% or more in at least one population, while the other 4 have a very low frequency of less than 3%. Some rarer alleles may exist, since the frequency threshold above which a given allele has a 95% likelihood to be detected with the sample size used is about 0.02. Some examples of allelic variation are given in Fig. 1.

Most Chi-2 tests of departure from Hardy-Weinberg expectations of genotype frequencies under panmixia are not significant. Only 8 populations for Got-2 and 23 populations for Acp were found to have a significant deficit of heterozygotes. Prx often shows an excess of rare homozygotes AA, the Chi-2 test cannot be applied because of the very low number of expected genotypes in this class.

Table 2. Summary of allele frequencies and population genetic statistics

Allele	Average freq.	Range in 60 pop.		No of pop.
PGI_a+	0.003	0	0.099	5
PGI_a	0.376	0.155	0.704	60
PGI_b	0.516	0.169	0.730	60
PGI_c	0.081	0	0.232	59
PGI_d	0.022	0	0.105	43
PGI_e	0.001	0	0.030	6
ACP_a+	0.002	0	0.057	6
ACP-a	0.460	0.204	0.780	60
ACP_b	0.455	0.144	0.728	60
ACP_c	0.048	0	0.241	55
ACP_d	0.035	0	0.148	49
ACP_e	0.001	0	0.023	7
GOT2_a+	0.004	0	0.063	14
GOT2_a	0.231	0.061	0.746	60
GOT2_b	0.761	0.183	0.939	60
GOT2_c	0.003	0	0.031	11
GOT3_a+	0.001	0	0.017	3
GOT3_a	0.034	0	0.147	52
GOT3_b	0.834	0.492	1.000	60
GOT3_c	0.109	0	0.333	58
GOT3_d	0.018	0	0.113	38
SOD_a	0.022	0	0.148	39
SOD_b	0.978	0.852	1.000	60
PRX_a	0.033	0	0.202	48
PRX_b	0.967	0.798	1.000	60
IDH_a	0.008	0	0.051	20
IDH_(b + c)	0.955	0.737	1.000	60
IDH_d	0.037	0	0.263	39
A mean No of alleles/locus	2.75	1.9	3.1	
P % polymorphic loci	63.6	42.9	100	
H Mean HW expected heterozygosity	0.270	0.200	0.359	

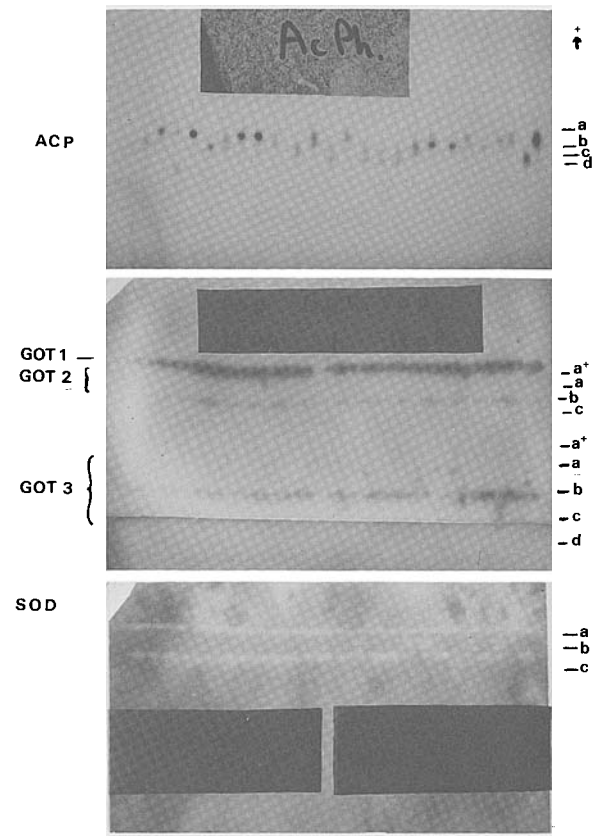

Fig. 1. Some examples of allelic variation for isozyme in perennial ryegrass populations.

Table 3 summarizes Wright's F statistics at each locus. Again Acp, Got-2 and Prx show the highest fixation indices F_{it} and F_{is}, which illustrate their deficits of heterozygotes. This discrepancy between Acp, Got-2, Prx and the other 4 loci may be accounted for by some misinterpretation of gel patterns, particularly for Acp which shows a poor resolution on the lithium-borate basic gel used. The presence of null alleles or the linkage of isozyme loci with the self-incompatibility system of *Lolium perenne* may also be involved (Cornish et al., 1980b). Therefore we used only the 4 loci Pgi, Got-3, Sod and Idh in averaging F statistics. The mean F_{st} value is 0.054, which gives an estimate of the average number of migrants between any pair of populations of 4.3. The among population differentiation can also be expressed as a proportion of the diversity index H (mean heterozygosity expected under panmixia) by $D_{st}/H = F_{st}/(1 - F_{st})$ (Nei, 1977). In this study, $D_{st}/H = 0.054/0.946 = 6\%$. Only 6% of the genetic diversity is accounted for by among population differentiation. Reciprocally, this means that 94% is due to the within population diversity (H illustrates both the allelic "richness" and the allelic "evenness" Brown, 1978).

Table 3. Wright's F statistics: overall fixation index F_{it}, within population F_{is}, between population F_{st}, between factor F_{xt} (e.g. between cluster) and between-population within factor (cluster ...) F_{sx}

				Hierarchical F statistics (Decomposition of F_{st})					
				X = agronomic cluster		X = admin. region		X = habitat	
Locus	F_{it}	F_{is}	F_{st}	F_{sx}	F_{xt}	F_{sx}	F_{xt}	F_{sx}	F_{xt}
Pgi-2	0.064	0.018	0.047	0.045	0.002	0.047	0	0.045	0.001
Acp-1	0.234	0.184	0.061						
Got-2	0.255	0.171	0.101						
Got-3	0.104	0.041	0.066	0.058	0.008	0.031	0.035	0.058	0.003
Sod-1	0.101	0.064	0.039	0.034	0.005	0.038	0.001	0.032	0.001
Prx-1	0.307	0.272	0.049						
Idh-1	0.096	0.032	0.062	0.056	0.006	0.047	0.015	0.050	0.007
Mean 4 loci	0.091	0.039	0.054	0.049	0.005	0.041	0.013	0.046	0.003

The hierarchical decomposition of F_{st} according to 3 factors of classification shows that none of these factors, namely agronomic cluster, administrative region or the type of habitat succeeded in explaining an important part of F_{st} by a between factor differentiation component. The only exception is administrative region for Got3, with a F_{sx} which accounts for about half the F_{st} value. This comes from the fact that some alleles of this locus show clinal trends and therefore are more abundant in the North and East regions. The theoretical relationships between the components is

$$1 - F_{st} = (1 - F_{sx})(1 - F_{xt}),$$

which for small values gives approximately:

$$F_{st} = F_{sx} + F_{xt}$$

This is well verified for x = agronomic cluster and x = administrative region. The small discrepancy for x = habitat ($F_{sx} + F_{xt} < F_{st}$) comes from a few missing data (7 populations for which habitat had not been recorded properly).

Geographic patterns and relationships with environmental factors

Two kinds of geographic patterns of variation have been observed for most alleles, except the rarest ones, as illustrated in Fig. 2.

1. Most rare alleles show very "erratic" patterns with apparently no spatial structure, as for example Pgi_a+ or Got-3_a. Some of these low frequency alleles however show some "patches": the populations having the highest frequency are neither randomly nor uniformly located, but are more or less close to each other e.g. Pgi_c, Acp_d or Idh_d.

2. Five alleles show a trend toward a North-South clinal variation: Acp_b and Prx_a are more frequent in the South, while Acp_a, Got3_c and Got3_d are more frequent in the North of France. Their frequencies are therefore associated with latitude and with the climatic variables which also have obvious North-South clines: average annual temperature (mean T), minimum temperature of the coldest month (min T), maximum temperature of the warmest month (max T) and potential evapotranspiration over the growth period (etp). These relationships are clearly shown in Fig. 3, which display the projection of allele frequency classes and climatic variable classes on the plan of the first two factors from the multiple factorial correspondence analysis, which explains 26% of the total inertia (this low value is due to the high number of variables considered, one for each class of allele frequency or climatic factor).

Discussion

Genetic diversity

The average genetic diversity indices P at the 0.99 criterium (63.6%), A (2.75), and H (0.270) fall close to the upper limit of the range reported by Hamrick et al. (1979) for outcrossed, wind pollinated, short-lived perennial, regionally distributed species. As pointed out by these authors, nonweedy species with a broad distribution may be expected to experience a great range of ecological

conditions, to have large population sizes, to have a more continuous distribution and a great potential for gene flow, which could lead to the maintenance of high levels of genetic variation. This seems to apply to the present study of ryegrass populations. Our diversity indices are very similar to those reported by Hayward & Mcadam (1977) for 9 perennial ryegrass varieties at 4 loci ($P = 75\%$, $A = 2.25$, $H = 0.267$) and to those observed by Hayward (1985) in 40 natural

(a)

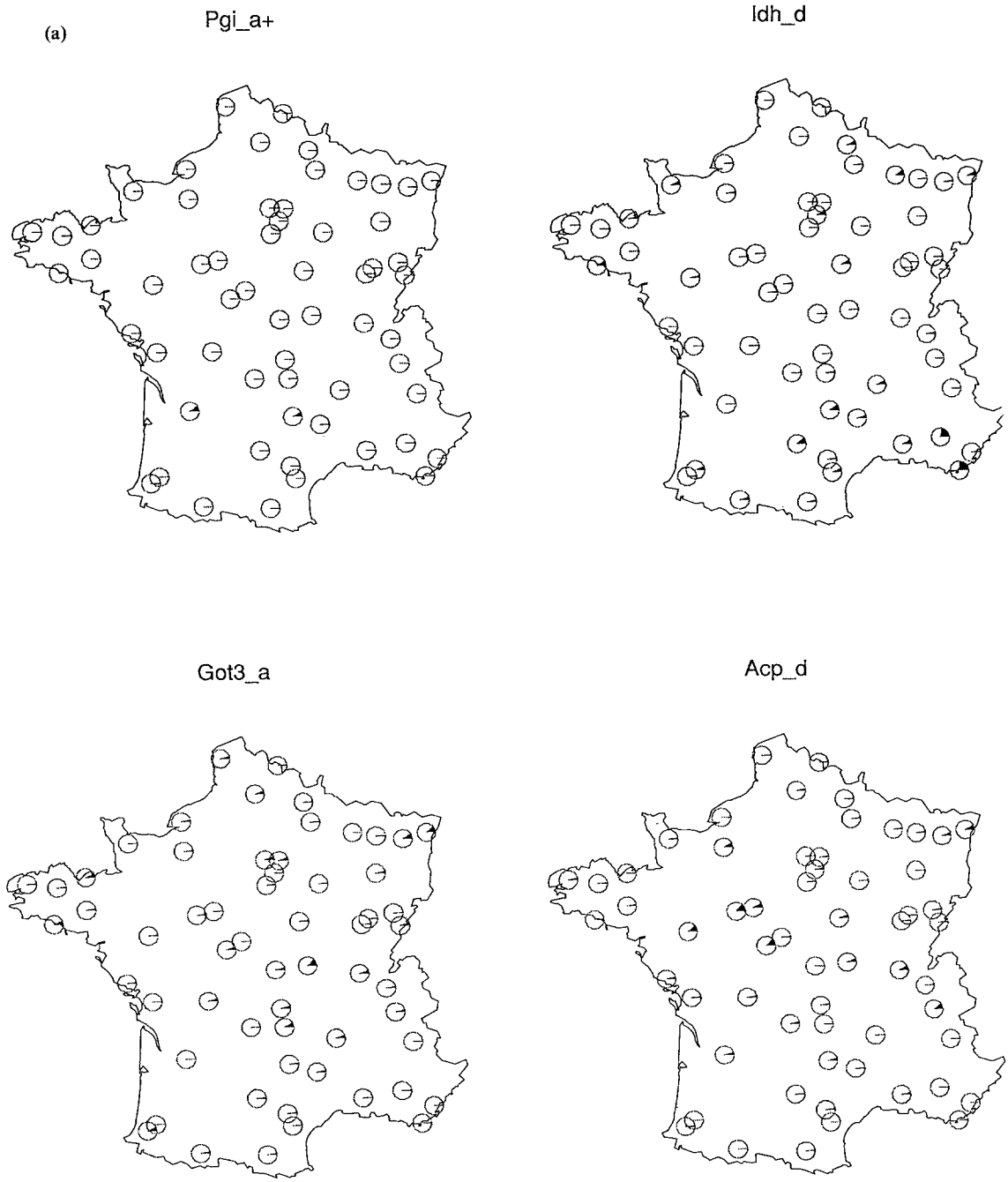

Fig. 2(a).

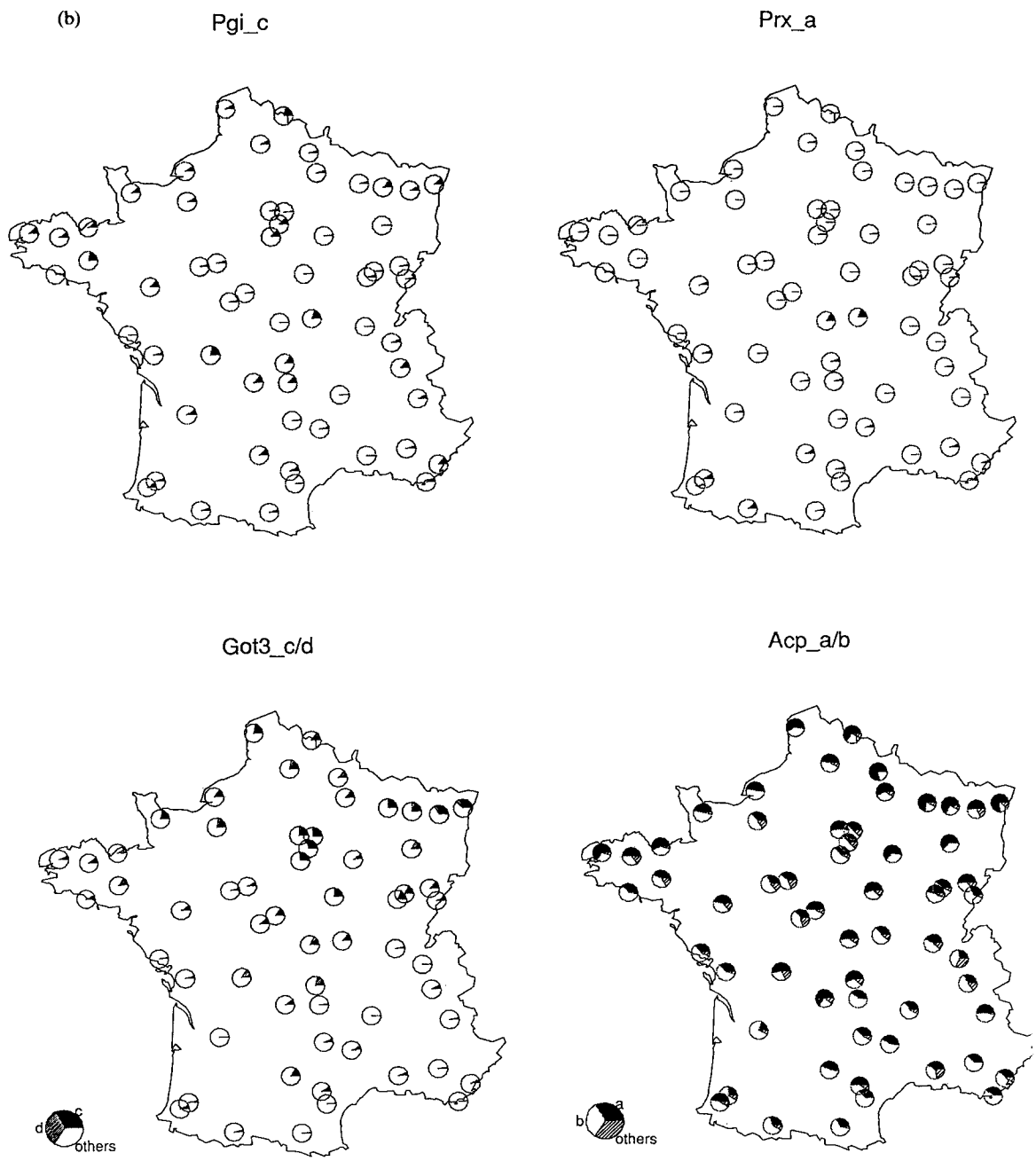

Fig. 2. Geographic patterns of variation for 10 alleles of isozymes in 60 populations of perennial ryegrass. Filled or shaded sectors are proportional to allele frequencies.

populations from Britain (A = 3.09 H = 0.372 on 5 loci). However, when compared to perennial ryegrass populations from Italy (Hayward, 1985; Arcioni et al., 1988), from North Spain (Oliveira & Charmet, 1989) or to Italian ryegrass (*Lolium multiflorum* Lam.) (Coleman, 1977), our sample of French populations of perennial ryegrass shows lower diversity indices. This may indicate that France is further from the Mediterranean center of origin of the genus *Lolium* than for example Italy or Spain.

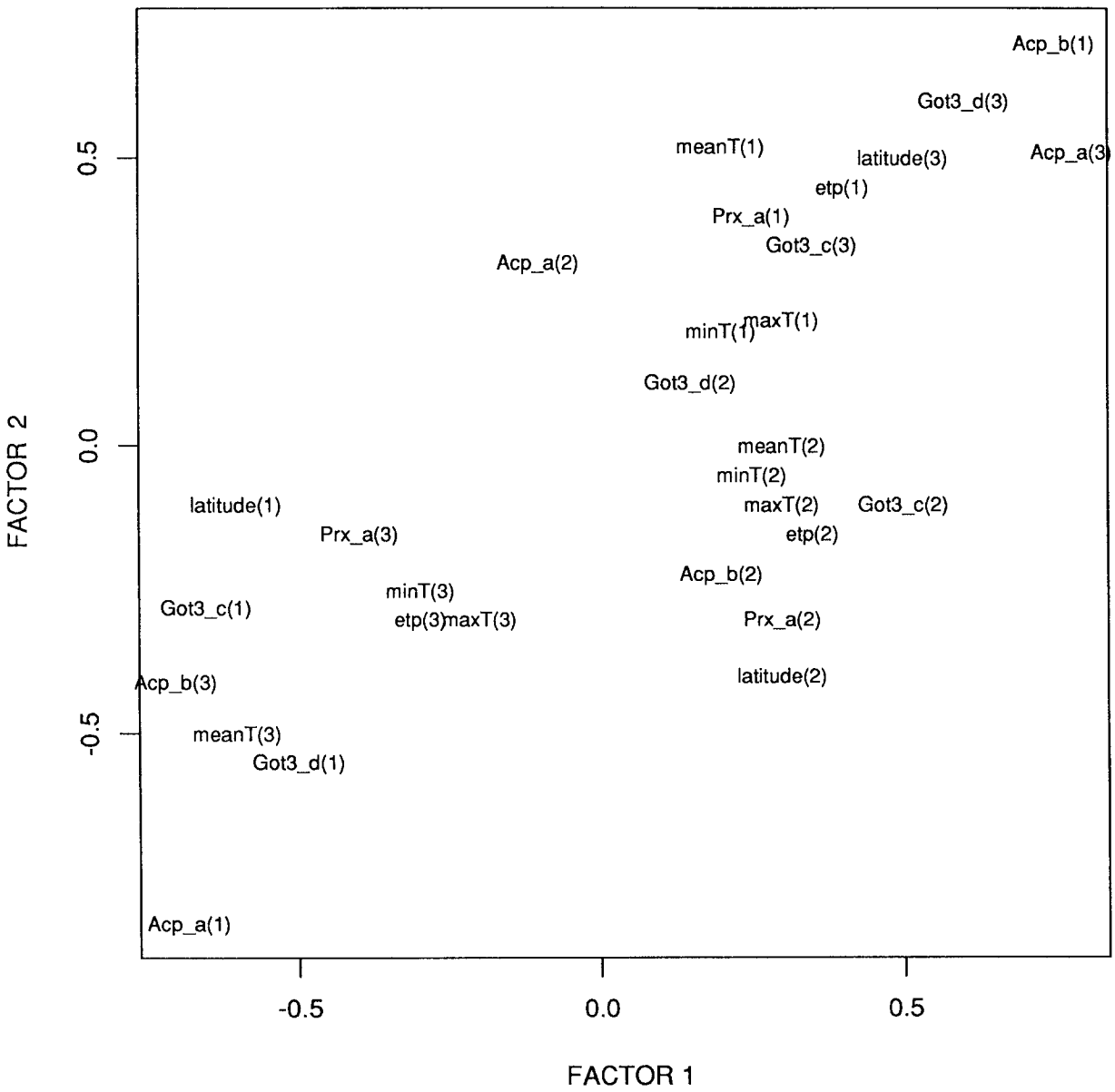

Fig. 3. Projection of allele frequency and climatic variables on the plan of the two factors of factorial correspondence analysis. The number in brackets indicates either the frequency class (1 = rare, 2 = intermediate, 3 = abundant) or the level of the climatic variable (1 = low, 2 = intermediate, 3 = high).

The geographic distribution of diversity indices does not highlight any particular region as having more diverse populations than the others.

Within population genetic structure

Although the present survey was not designed to study fine scale within population substructuring, some comments can be made from the F_{is} values. The average fixation index, F_{is}, on 60 populations is about 0.04, which is the same value as that reported by Hayward & Mcadam (1977) for *Lolium perenne*, and half the value observed at seedling stage in *Lolium multiflorum* by Allard et al. (1977). As stated in the results section, the F_{is} value is consistently greater for Acp, Got-2 and Prx loci,

and lower for Pgi. Ostergaard et al. (1985) have reported the presence of null alleles at the Acp locus. Such a null allele of frequency f will bias the estimate of F_{is} by $2f/(1-f)$ (Brown 1978). For Acp this bias is about 0.12, which would give $f = F_{is}/(2-F_{is}) = 0.06$. The frequency of null homozygotes, if viable, is thus expected to be $(0.06)^2 = 0.004$, which has a probability of 0.33 to be detected in a sample of 100 diploid plants. We would thus have expected to observe at least one null homozygote in 20 populations out of 60, which was not the case. Null homozygotes at ACP have only been observed in selfed progenies (unpublished data), but never in natural populations. We can conclude that null alleles are not the only cause of bias in F_{is} estimates from Acp, Got-2 or Prx. Since the interpretation of these loci is sometimes uncertain, we have excluded them for averaging F_{is} across loci.

Although most Chi-2 tests for departure from Hardy-Weinberg proportions are not significant, Lewontin & Cockerham (1959) noted the relative insensitivity of this test, due to large sampling errors. Since the F_{is} value for the 60 populations studied is consistently positive, we must assume that F_{is} is significant and try to explain the corresponding deficit of heterozygotes. This deficit may have two causes:

Partial selfing: in the case of mixed mating system, with a constant outcrossing rate t, the within population fixation index is $F_{is} = (1-t)/(1+t)$ (Brown, 1979). The observed F_{is} of 0.04 would lead us to assume $t = 0.92$, or reciprocally a selfing rate of 8%. Although Allard et al. (1977) postulated a selfing rate of 17% to be responsible for the deficit of heterozygotes at the seedling stage in Italian ryegrass, we believe this is very unlikely in perennial ryegrass. In a selfing experiment on a similar set of populations (not published), about 70% of the bagged plants were completely self-sterile and most of the others gave less than one seed per spike. Only 4 plants out of 250 produced more than 10 seeds. Therefore we can consider the two locus self incompatibility system of perennial ryegrass as quite complete (Cornish et al., 1980a), and assume $t = 1$.

Restricted neighborhood size and Wahlund effect: the native population collected at a given site may comprise a mosaic of neighborhoods, i.e. circles of individuals which are on average more genetically related than two random members of the entire local population. Mating is panmictic within a neighborhood, and only a limited gene flow can occur between neighborhoods through pollen or seed transport. From Kirby (1975), the expected fixation index of a sample bulked from many neighborhoods is $F_{is} = (1-4m)/(1+4mN)$, where N is the effective size of each neighborhood and m the proportion of migrants between neighborhoods at each generation. Although we do not yet have reliable estimates of N in perennial ryegrass, the observed value of F_{is} can be obtained for reasonable estimates such as $N = 100$ and $m = 0.05$. So this cause is more likely to explain the deficit of heterozygotes in the case of *Lolium perenne* L.

Between populations differentiation

Wright's F_{st} for multiallelic loci being equivalent to the Nei's G_{st} measure of population differentiation, we can compare our results with other published values of either F_{st} or G_{st}.

In this study, a mean F_{st} of 0.054 has been observed. Compared to the list of Hamrick et al. (1979), this value is in the lowest range for outbreeders, and is more similar to that of tree species (Tigerstedt, 1974, Muona, 1989, Comps et al., 1990) or to that of *Plantago lanceolata* L. (Van Dijk et al., 1988), another wind-pollinated, self incompatible perennial herb from grasslands than to other herbaceous plants such as *Clarkia* (Gottlieb, 1974) or *Phlox* (Levin, 1977). In a more recent review of the literature, Hamrick& Godt (1989) reported a mean G_{st} of 0.098 for 134 outcrossing-wind species. Our set of ryegrass populations show a differentiation level of about half this value. This can be explained by the fact that it originates from only a part of the whole distribution area of this species. Perennial ryegrass from France appears to show little among-population differentiation. Assuming an infinite island model would lead to the supposition that each population exchange on average 4.3 migrants per generation with the hypothesised continent. This point will be further discussed in the next section.

A practical consequence of this low differentiation for genetic resources management could be that a small number of populations would be sufficient to preserve most of the genetic diversity.

This diversity could be measured either by mean heterozygosity or by the mean number of alleles, since most alleles are common and widespread. However this conclusion only applies for isozymic variation and cannot be generalized to other traits.

Geographic structure of isozyme polymorphism

Some alleles show clinal patterns of geographic variation, the most illustrative being that of the allele "c" of Got-3, which shows a clinal trend from North-East to South-West. This cline could be interpreted either as a consequence of selection or as a consequence of migration from a North-Eastern source of populations rich in Got3_c towards the South-West. Further isozyme surveys on a broader scale are needed to support the latter hypothesis. Although less clear, the pattern of Acp_a/b also shows a clinal trend, from South to North.

Some of the rare alleles such as Pgi-c, Acp_d or Idh_d show erratic patterns, with however, some patches which could be the consequence of the diffusion by migration from a central "source". A possible cause of locally differentiated patches could be genetic drift. Genetic drift may be due to bottlenecks caused by founding of new populations by a small number of individuals after local extinctions. If such events are quite rare and recent, this could explain the occurrence of locally differentiated populations within a background of undifferentiated ones, before gene flow succeeds in homogenizing allele frequencies again. Obviously further experimental work or computer simulation would be needed to confirm these assumptions.

Most other loci show erratic patterns, where no geographic structuring can be observed (e.g. Pgi_a+ or Got3_a), or are more or less homogeneously distributed over a large area, as for PGI_a/b and GOT2_b (not represented in Fig. 2). As reported by Sokal (1986), such an absence of geographic structure could be explained either by panmixia over a whole country or by a uniform selection pressure. This latter hypothesis is less plausible, since most isozymes are generally regarded as selectively neutral. Moreover, most of them are polymorphic, which makes it necessary to suppose a sophisticated form of balancing selection for each locus.

The panmixia hypothesis is supported by the high estimate of average gene flow among populations. Such a high gene flow among populations might be accounted for by long-distance seed transport by animals (e.g. birds, horses), or even pollen transport by wind if we assume very large neighborhood sizes in a continuous population model. Another explanation, proposed by Alden & Loopstra (1987) for *Picea glauca* (Molich) Voss in Alaska, is that the period since the colonization has been too short (taking into account the effective size of populations, generation length etc.) to allow populations to differentiate clearly. This might apply to the alleles Pgi_a+ and Got3_a, which are rare and may evolve from recent mutations.

Relationships with climatic factors

A clear correspondence has been established between the frequency of five alleles involving three loci and a set of climatic variables from the collection sites (Fig. 3). These relationships reflect a clinal variation of both the allelic frequency and the average temperature in different seasons. Whether these alleles are actually selected by specific climatic conditions or simply vary clinally with latitude is a difficult question. Populations sampled on several independent (i.e. distant) climatic gradients should be examined to support the selection hypothesis.

In tetraploid *Dactylis glomerata* L. Lumaret (1984) found a wide scale cline from Scotland to North Africa for several Got-1 alleles, whose frequencies were obviously correlated with climatic factors. Moreover the same correlations were found on two small scale transects in two distant countries, and the different alleles showed different patterns of in vitro activity according to temperature. Lumaret concluded that allelic diversity probably confers a selective advantage (better fitness), especially in transitional climatic areas.

In the conifer Norway spruce, Bergmann (1978) also reported a clinal variation of an Acp allele to be associated with two similar climatic gradients.

In perennial ryegrass, Arcioni et al. (1988) reported correlations between Pgi_b and Pgi_d alleles and the temperature and summer rainfall in Italy, resulting in a correlation with latitude. In the present study, Pgi alleles are not correlated with any climatic variable. The selection hypothesis is

therefore doubtful, and the cline observed in Italy may be explained by introgression from *Lolium rigidum* Gaud., an annual species with a high frequency of PGI_d, which is more abundant in Southern Italy.

Hayward & Mcadam (1988) investigated the effect of selection on isozymic variants on yield and flowering time in three cultivars of perennial ryegrass. They explained most of the significant correlations by the founder principle, rather than by co-selection events. Recently, in an attempt to find Quantitative Traits Loci (QTL) using isozymes in *Lolium perenne*, Humphreys (1992) observed a consistent association between water soluble carbohydrate content and genotype at the PGI locus. Some association was also detected between genotype at the Acp locus and heading date. Since traits such as flowering time or yield are adaptative, they can be selected by climatic factors (e.g. early flowering in warm-dry locations). The alleles which are closely linked with such adaptative traits may be selected by the same climatic factors, even if they are themselves neutral.

Country-scale clinal patterns of variation have also been reported in two species of land snails (Ochman et al., 1987), but latitudinal changes in allele frequency in British populations were reversed in France and Spain. The authors concluded that there was no strong evidence for the action of selection. More than one cline and consistent associations between alleles and climatic traits should therefore be identified before to explain a clinal pattern as a result of natural selection.

In human populations, many alleles show clinal patterns (see for a review Sokal et al., 1989). They are mostly ascribed to successive waves of eastern migrants into Europe. Can similar migration events explain the clines observed for Acp_a/b, Got3_c/d and Prx_a alleles?

Clearly it would be useful to make further studies to demonstrate the possible effect of selection on isozymes, as for example a survey on different scales: large or small transect showing similar gradients of climatic variation. The availability of additional loci would enable us to employ multivariate methods such as principal components analysis to investigate the migration hypothesis as proposed by Rendine et al. (1986).

Conclusions

This survey of electrophoretic variation in a collection of natural populations of perennial ryegrass from France, although based on few loci, confirms that perennial ryegrass show population genetics statistics which fall in the range of values observed in other species of similar life-cycle characteristics. Particularly the fact that most of the total gene diversity is accounted for by the within population variation component agrees with Hamrick et al. (1979). This suggests that sampling few populations for genetic resources could be sufficient to preserve most of the species' variability for isozymic variation. Given the high genetic variation and low fixation indices, inbreeding depression could be important. Therefore large effective population size must be used to avoid inbreeding depression and genetic drift. This could be achieved either by *in situ* conservation, for example in ecological reserves, or by *ex situ* conservation, providing a sufficient number of plants is used for seed regeneration.

Although limited in this collection, the among population component of differentiation may be larger on a wider geographic range. Moreover, genetic resources are primarily of use for plant breeders, who look for adaptative traits e.g. tolerance to stress and growth potential. Therefore the sampling and conservation of natural populations should be oriented on the amount of diversity for adaptative traits, and not only based on neutral marker studies. This was taken into account in the proposal for the formation of a "core collection" for *Lolium perenne* L. (Balfourier & Charmet, 1993). Although rather partial because of the limited geographic range, this study has indicated some clinal trends in allelic variation, the origin of which is still uncertain. These trends may be due to large scale migration or expansion from a middle-East center of origin, as observed for chestnut (Villani et al., 1991); further studies are being carried out to confirm this hypothesis. In such a case, the collection and preservation of native populations from this presumed "center of origin" should be recommended. In the case of *in situ* conservation, suitable sites should be found both in Middle East and in western countries. Moreover, as suggested by (Eguiarte et al., 1992), other sites, scarcely located throughout the

"migration route", should also be preserved. These "witnesses" of past migration may allow gene flow among the main *in situ* conservation sites and insure that the evolution of perennial ryegrass will continue.

Acknowledgements

This work was supported by a grant no 88 R 0704 from the French Ministry of Research and Technology.

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